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=> s antibod?
L1      2374358 ANTIBOD?

=> s l1 and "GPVI
MISMATCHED QUOTE 'AND "GPVI'
Quotation marks (or apostrophes) must be used in pairs,
one before and one after the expression you are setting
off or masking.

=> s l1 and "GPVI"
L2          167 L1 AND "GPVI"

=> s l2 and monoclonal
L3           93 L2 AND MONOCLONAL

=> s l3 and glycoprotein VI
L4           61 L3 AND GLYCOPROTEIN VI

=> s l4 and platelet
L5           61 L4 AND PLATELET

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PROCESSING COMPLETED FOR L5
L6           21 DUP REMOVE L5 (41 DUPLICATES REMOVED)

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L6    ANSWER 1 OF 21      MEDLINE                                  DUPLICATE 1
2002079876  Document Number: 21659770.     PubMed ID: 11723134.   The
     platelet receptor GPVI mediates both adhesion and
     signaling responses to collagen in a receptor density-dependent fashion.
     Chen Hong; Locke Darren; Liu Ying; Liu Changdong; Kahn Mark L. (Department
     of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104,
     USA. ) JOURNAL OF BIOLOGICAL CHEMISTRY, (2002 Jan 25) 277 (4) 3011-9.
     Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States.
     Language: English.
Ab   The platelet response to collagen is a primary event in
     hemostasis and thrombosis, but the precise roles of the numerous
     identified platelet collagen receptors remain incompletely
     defined. Attention has recently focused on glycoprotein
     VI (GPVI), a receptor that is expressed on
     platelets in association with a signaling adapter, the Fc receptor
     gamma chain (Fc Rgamma). Genetic and pharmacologic loss of GPVI
     function results in loss of collagen signaling in platelets, but
     studies to date have failed to demonstrate that GPVI-Fc Rgamma
     expression is sufficient to confer collagen signaling responses. These
     results have led to the hypothesis that collagen responses mediated by
     GPVI-Fc Rgamma may require the collagen-binding integrin
     alpha2beta1 as a co-receptor, but this model has not been supported by a
     recent study of mouse platelets lacking alpha2beta1. In the
     present study we have used a novel anti-GPVI monoclonal
     antibody to measure the level of GPVI on human
     platelets and to guide the development of GPVI
     -expressing cell lines to assess the role of GPVI in mediating
     platelet collagen responses. GPVI receptor density on
     human platelets appears tightly regulated, is independent from
     the level of alpha2beta1 expression, and significantly exceeds that on
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previously characterized **GPVI**-expressing RBL-2H3 cells. Using newly generated **GPVI**-expressing RBL-2H3 cells with receptor densities equivalent to that on human **platelets**, we demonstrate that **GPVI** expression confers both adhesive and signaling responses to collagen in a graded fashion that is proportional to the **GPVI** receptor density. These results resolve some of the conflicting data regarding **GPVI**-collagen interactions and demonstrate that 1) **GPVI**-Fc R gamma expression is sufficient to confer both adhesion and signaling responses to collagen, and 2) **GPVI**-mediated collagen responses are receptor density-dependent at the receptor levels expressed on human **platelets**.

L6 ANSWER 2 OF 21 MEDLINE DUPLICATE
2002[illegible] Document Number: 22[illegible]. PubMed ID: 12117414. Differential effects of reduced **glycoprotein VI** levels on activation of murine **platelets** by **glycoprotein VI** ligands. Snell Daniel C; Schulte Valerie; Jarvis Gavin E; Arase Kanoh; Yokozai Daiju; Saito Takashi; Watson Steve P; Nieswandt Bernhard. Department of Pharmacology, University of Oxford, Mansfield Road, Oxford OX1 3QT, U.K. BIOCHEMICAL JOURNAL, (2002 Nov 15) 368 (Pt 1) 293-300. Journal code: 2984726R. ISSN: 0264-6021. Pub. country: England: United Kingdom. Language: English.

AB We have investigated the effects of decreased levels of the complex between **glycoprotein VI** (**GPVI** and the Fc receptor gamma chain (FcRgamma) on responses to collagen and **GPVI**-specific ligands in murine **platelets**. We show that levels of **GPVI**-FcRgamma of the order of 50% and 20% of wild-type levels induce 2- and 5-fold shifts to the right respectively in the dose-response curve for aggregation in response to collagen, the snake toxin convulxin and the **monoclonal antibody** JAQ1. In addition, there is a delay in the onset of aggregation in response to collagen. In contrast, the stimulation of protein tyrosine phosphorylation by collagen (as measured after 120 s) and adhesion to a collagen-coated surface under static conditions were unaffected in **platelets** with 50% and 20% of wild-type levels of **GPVI**. In contrast, responses to a collagen related peptide (CRP), made up of repeat glycine-proline-hydroxyproline motifs, were markedly inhibited and abolished in **platelets** expressing 50% and 0% of wild-type levels of **GPVI** respectively. We suggest that the marked effect of a reduction in **GPVI** levels on the CRP-induced activation of **platelets** is due to the multivalent nature of CRP and the fact that **GPVI** is its sole receptor on **platelets**. Thus it appears that the interaction of CRP with **GPVI** is determined by a combination of affinity and avidity. The observation that collagen does not behave like CRP in **platelets** expressing reduced levels of **GPVI**, even in the combined presence of blocking **antibodies** against integrin alpha2beta1 and GPV, suggests that collagen has a greater affinity than CRP for **GPVI**, and/or that other receptors are involved in its binding to **platelets**. The clinical significance of these results is discussed.

L6 ANSWER 3 OF 21 CAPLUS COPYRIGHT 2002 ACS
2001:165145 Document No. 134:217[illegible] **Platelet** membrane **glycoprotein VI** (**GPVI** cDNA and protein sequences, and therapeutic uses thereof. Tandon, Narendra; Sun, Bing; Nakamura, Takashi; Yamamoto, Naomasa Otsuka Pharmaceutical Co., Ltd., Japan). PCT Int. Appl. WO 2001016321 A1 2001308, 71 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK,

ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US23975 20000901. PRIORITY: US 1999-PV152197 19990901; US 1999-PV153251 19991007.

AB The present invention comprises a method of purifying **platelet** membrane **glycoprotein VI** (**GPVI**), **GPVI** peptides, cDNA and protein sequence, and methods for using **GPVI** and **antibodies** directed against **GPVI**. It was shown that the extracellular domain of **GPVI** has potent anti-thrombotic activity. The invention comprises methods of inhibiting thrombosis by inhibiting **platelet** aggregation or **platelet** activation using **antibodies** directed against **GPVI**, or **GPVI** protein, in particular, the extracellular domain of **GPVI**.

L6 ANSWER 4 OF 21 CAPLUS COPYRIGHT 2002 ACS

2001:[illegible] Document No. 134:31[illegible]5 **Glycoprotein VI** cDNA and protein from human and murine blood **platelets** and their diagnostic and therapeutic applications. Barnfield, Samantha J.; Villeval, Jean-Luc; Jandrot-Perrus, Martine; Watchmaker, William; Gill, Davinder Singh; Qian, Ming Dong; Kunicsky, Hillar. Millennium Pharmaceuticals, Inc., USA). PCT Int. Appl. WO 2001006861 A1 20010201, 127 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US21815 20000630. PRIORITY: US 1999-145468 19990630; US 1999-447814 19991206; US 2000-503387 20000214.

AB The invention provides isolated cDNA mols. and polypeptide mols. that encode human and murine **glycoprotein VI**, a **platelet** membrane glycoprotein that is involved **platelet**-collagen interactions. The protein initially designated TANGO 268 represents the **platelet**-expressed collagen receptor **glycoprotein VI** (**GPVI**) based on the following evidence: (1) the glycosylated mol. wts. of TANGO 268 and **GPVI** are identical or similar; (2) both are recognized by anti-**GPVI** **antibodies** and bind to convulxin; (3) both are preferentially expressed in megakaryocytic cells; 4) both is predicted to have a single N-glycosylation site; (5) the mol. mass of **GPVI** upon N- and O-linked glycosylation is approx.62 kDa, that of **GPVI**; 6) two Ig-like domains in TANGO 268 indicates interaction with FcR.gamma.; (7) the absence of a large intracytoplasmic tail suggests that this membrane-bound glycoprotein has no signaling role but assocs. with another member of the Ig family; and (8) TANGO 268 has a charged arginine residue in the transmembrane domain which is also predicted to be present in **GPVI**. The human gene for **GPVI** was mapped on radiation hybrid panels to the long arm of chromosome 19, in the region 19q13, syntenic to mouse chromosome 7. The invention also provides antisense nucleic acid mol., expression vectors contg. the nucleic acid mols. of the invention, host cells into which the expression vectors have been introduced, and non-human transgenic animals in which a nucleic acid mol. of the invention has been introduced or disrupted. The invention still further provides isolated polypeptides, fusion polypeptides, antigenic peptides and **antibodies**. Diagnostic, screening and therapeutic methods utilizing compns. of the invention are also provided.

L6 ANSWER 5 OF 21 MEDLINE DUPLICATE 3

20014[illegible]6540 Document Number: [illegible]559356. Pubmed ID: 11544165. A novel viper venom metalloproteinase, alborhagin, is an agonist at the **platelet** collagen receptor **GPVI**. Andrews R K; Gardiner E E; Asazuma N;

Berlanga O; Tulasne D; Nieswandt B; Smith A I; Berndt M C; Watson S P.
Hazel and Pip Appel Vascular Biology Laboratory and the Peptide Biology
Laboratory, Baker Medical Research Institute, Melbourne 3008, Australia..
rkandrews@hotmail.com . JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Jul 27)
276 (30) 28092-7. Journal code: 2985121R. ISSN: 0021-9258. Pub. country:
United States. Language: English.

AB The interaction of **platelet** membrane **glycoprotein**
VI (**GPVI**) with collagen can initiate
pathophysiological thrombus formation. The viper venom C-type lectin
family proteins convulxin and alboaggregin-A activate **platelets**
by interacting with **GPVI**. In this study, we isolated from
white-lipped tree viper (Trimeresurus albolabris) venom, alborhagin, which
is functionally related to convulxin because it activates
platelets but is structurally different and related to venom
metalloproteinases. Alborhagin-induced **platelet** aggregation
(EC50, [illegible] microg/ml) was inhibitable by an anti alphaIIbbeta3
antibody, CRC64, and the Src family kinase inhibitor PP2,
suggesting that alborhagin activates **platelets**, leading to
alphaIIbbeta3-dependent aggregation. Additional evidence suggested that,
like convulxin, alborhagin activated **platelets** by a mechanism
involving **GPVI**. First, alborhagin- and convulxin-treated
platelets showed a similar tyrosine phosphorylation pattern,
including a similar level of phospholipase Cgamma2 phosphorylation.
Second, alborhagin induced **GPVI**-dependent responses in
GPVI-transfected K562 and Jurkat cells. Third,
alborhagin-dependent aggregation of mouse **platelets** was
inhibited by the anti-**GPVI monoclonal antibody**
JAQ1. Alborhagin had minimal effect on convulxin binding to **GPVI**
-expressing cells, indicating that these venom proteins may recognize
distinct binding sites. Characterization of alborhagin as a **GPVI**
agonist that is structurally distinct from convulxin demonstrates the
versatility of snake venom toxins and provides a novel probe for
GPVI-dependent **platelet** activation.

L6 ANSWER 6 OF [illegible] MEDLINE DUPLICATE 4
2001398778 Document Number: 2132[illegible]. PubMed ID: 1135[illegible]. Rhodocytin
(aggretin) activates **platelets** lacking alpha(2)beta(1) integrin,
glycoprotein VI, and the ligand-binding domain of
glycoprotein Ibalpha. Bergmeier W; Bouvard D; Eble J A; Mokhtari-Nejad R;
Schulte V; Zirngibl H; Brakebusch C; Fassler R; Nieswandt B. (Department
of Molecular Oncology, General Surgery, Witten/Herdecke University,
Arrenbergerstr. [illegible], Haus 10, 42117 Wuppertal, Germany. JOURNAL OF
BIOLOGICAL CHEMISTRY, (2001 Jul 6) 276 ([illegible]) 25121-6. Journal code:
2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Although alpha(2)beta(1) integrin (glycoprotein Ia/IIa) has been
established as a **platelet** collagen receptor, its role in
collagen-induced **platelet** activation has been controversial.
Recently, it has been demonstrated that rhodocytin (also termed aggretin),
a snake venom toxin purified from the venom of Calloselasma rhodostoma,
induces **platelet** activation that can be blocked by
monoclonal antibodies against alpha(2)beta(1) integrin.
This finding suggested that clustering of alpha(2)beta(1) integrin by
rhodocytin is sufficient to induce **platelet** activation and led
to the hypothesis that collagen may activate **platelets** by a
similar mechanism. In contrast to these findings, we provided evidence
that rhodocytin does not bind to alpha(2)beta(1) integrin. Here we show
that the Cre/loxP-mediated loss of beta(1) integrin on mouse
platelets has no effect on rhodocytin-induced **platelet**
activation, excluding an essential role of alpha(2)beta(1) integrin in
this process. Furthermore, proteolytic cleavage of the 45-kDa N-terminal
domain of glycoprotein (GP) Ibalpha either on normal or on beta(1)-null
platelets had no significant effect on rhodocytin-induced
platelet activation. Moreover, mouse **platelets** lacking

both alpha(2)beta(1) integrin and the activating collagen receptor **GPVI** responded normally to rhodocytin. Finally, even after additional proteolytic removal of the 45-kDa N-terminal domain of GPIbalpha rhodocytin induced aggregation of these **platelets**. These results demonstrate that rhodocytin induces **platelet** activation by mechanisms that are fundamentally different from those induced by collagen.

L6 ANSWER 7 OF 21 MEDLINE DUPLICATE 5
2001304773 Document Number: 21293084. PubMed ID: 11237424. Aggretin, a heterodimeric C type lectin from Calloselasma rhodostoma (malayan pit viper), stimulates **platelets** by binding to alpha 2beta 1 integrin and glycoprotein Ib, activating Syk and phospholipase Cgamma 2, but does not involve the **glycoprotein VI**/Fc receptor gamma chain collagen receptor. Navdaev A; Clemetson J M; Polgar J; Kehrel b B; Glazer M; Magnenat E; Wells T N; Clemetson K J. (Theodor Kocher Institute, University of Berne, Freiestrasse 1, CH-3012 Berne, Switzerland.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Jun 15) 276 (24) 21167-9. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Aggretin, a potent **platelet** activator, was isolated from Calloselasma rhodostoma venom, and 30-amino acid N-terminal sequences of both subunits were determined. Aggretin belongs to the heterodimeric snake C-type lectin family and is thought to activate **platelets** by binding to **platelet** glycoprotein alpha(2)beta(1). We now show that binding to glycoprotein (GP) Ib is also required. Aggretin-induced **platelet** activation was inhibited by a **monoclonal antibody** to GPIb as well as by **antibodies** to alpha(2)beta(1). Binding of both of these **platelet** receptors to aggretin was confirmed by affinity chromatography. No binding of other major **platelet** membrane glycoproteins, in particular **GPVI**, to aggretin was detected. Aggretin also activates **platelets** from Fc receptor gamma chain (Fcgamma)-deficient mice to a greater extent than those from normal control mice, showing that it does not use the **GPVI** Fcgamma pathway. **Platelets** from Fcgamma-deficient mice expressed fibrinogen receptors normally in response to collagen, although they did not aggregate, indicating that these **platelets** may partly compensate via other receptors including alpha(2)beta(1) or GPIb for the lack of the Fcgamma pathway. Signaling by aggretin involves a dose-dependent lag phase followed by rapid tyrosine phosphorylation of a number of proteins. Among these are p72(SYK), p125(FAK), and PLCgamma2, whereas, in comparison with collagen and convulxin, the Fcgamma subunit neither is phosphorylated nor coprecipitates with p72(SYK). This supports an independent, GPIb- and integrin-based pathway for activation of p72(SYK) not involving the Fcgamma receptor.

L6 ANSWER 8 OF 21 MEDLINE DUPLICATE 6
2001370661 Document Number: 21226781. PubMed ID: 11278467. Expression and function of the collagen receptor **GPVI** during megakaryocyte maturation. Lagrue-Lak-Hal A H; Debili N; Hindbury G; Lecut C; Le Couedic J P; Villeval J L; Jandrot-Perrus M; Vainchenker W. (INSERM E9907, Faculte Xavier Bichat, 75870 Paris Cedex 18, Paris, France.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 May 4) 276 (18) 15316-25. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB In this report, the expression and function of the **platelet** collagen receptor **glycoprotein VI** (**GPVI**) were studied in human megakaryocytes during differentiation and maturation of mobilized blood and cord blood derived CD34(+) cells. By flow cytometry, using an anti-**GPVI monoclonal antibody** or convulxin, a **GPVI**-specific ligand, **GPVI** was detected only on CD41(+) cells including some CD41(+)/CD34(+) cells, suggesting expression at a stage of differentiation

similar to CD41. These results were confirmed at the mRNA level using reverse transcription-polymerase chain reaction. **GPVI** expression was low during megakaryocytic differentiation but increased in the more mature megakaryocytes (CD41(high)). As in **platelets**, megakaryocyte **GPVI** associates with the Fc receptor gamma chain (FcRgamma). The FcR gamma chain was detected at the RNA and protein level at all stages of megakaryocyte maturation preceding the expression of **GPVI**. The other collagen receptor, alpha 2)beta(1) integrin (CD49b/CD29), had a pattern of expression similar to **GPVI**. Megakaryocytic **GPVI** was recognized as a 55-kDa protein by immunoblotting and ligand blotting, and thus it presented a slightly lower apparent molecular mass than **platelet GPVI** (58 kDa). Megakaryocytes began to adhere to immobilized convulxin via **GPVI** after only 3-10 days of culture, at a time when megakaryocytes were maturing. At this stage of maturation, they also adhered to immobilized collagen by alpha 2)beta 1) integrin-dependent and -independent mechanisms. Convulxin induced a very similar pattern of protein tyrosine phosphorylation in megakaryocytes and **platelets** including Syk, FcRgamma, and PLC gamma2. Our results showed that **GPVI** is expressed early during megakaryocytic differentiation but functionally allows megakaryocyte adherence to collagen only at late stages of differentiation when its expression increases.

L6 ANSWER 9 OF 21 MEDLINE DUPLICATE 7

20013569 Document Number: 21246628. PubMed ID: 11389621. Evidence for cross-talk between **glycoprotein VI** and Gi-coupled receptors during collagen-induced **platelet** aggregation.

Nieswandt B; Bergmeier W; Eckly A; Schulte V; Ohlmann P; Cazenave J P; Cirnaru M; Offermanns S; Gachet C. (Department of Molecular Oncology, General Surgery, Witten/Herdecke University, Arrenbergerstrasse 20, 42117 Wuppertal, Germany.. nieswand@klinikum-wuppertal.de) . BLOOD, (2001 Jun 15) 97 (12) 3829-35. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Collagen-induced **platelet** aggregation is a complex process and involves synergistic action of integrins, immunoglobulin (Ig)-like receptors, G-protein-coupled receptors and their ligands, most importantly collagen itself, thromboxane A(2) (TXA(2)), and adenosine diphosphate (ADP). The precise role of each of these receptor systems in the overall processes of activation and aggregation, however, is still poorly defined. Among the collagen receptors expressed on **platelets**, glycoprotein (GP) VI has been identified to play a crucial role in collagen-induced activation. **GPVI** is associated with the FcRgamma chain, which serves as the signal transducing unit of the receptor complex. It is well known that clustering of **GPVI** by highly specific agonists results in **platelet** activation and irreversible aggregation, but it is unclear whether collagen has the same effect on the receptor. This study shows that **platelets** from Galphaq-deficient mice, despite their severely impaired response to collagen, normally aggregate on clustering of **GPVI**, suggesting this not to be the principal mechanism by which collagen activates **platelets**. On the other hand, dimerization of **GPVI** by a **monoclonal antibody** (JAQ1), which by itself did not induce aggregation, provided a sufficient stimulus to potentiate **platelet** responses to Gi-coupled, but not Gq-coupled, agonists. The combination of JAQ1 and adrenaline or ADP, but not serotonin, resulted in alpha(IIb)beta(3)-dependent aggregation that occurred without intracellular calcium mobilization and shape change in the absence of Galphaq or the P2Y(1) receptor. Together, these results provide evidence for a cross-talk between (dimerized) **GPVI** and Gi-coupled receptors during collagen-induced **platelet** aggregation. (Blood. 2001;97:3829-3835)

L6 ANSWER 10 OF 21 MEDLINE

2001272329 Document Number: 21231159. PubMed ID: 11321578.
Glycoprotein VI but not alpha2beta1 integrin is essential for **platelet** interaction with collagen. Nieswandt B; Brakebusch C; Bergmeier W; Schulte V; Bouvard D; Mokhtari-Nejad R; Lindhout T; Heemskerk J W; Zirngibl H; Fassler R. (Department of Molecular Oncology, General Surgery, Witten Herdecke University, 42117 Wuppertal, Germany.. nieswandt@klinikum-wuppertal.de . EMBO JOURNAL, (2001 May 1) 20 9 2120-30. Journal code: 8208664. ISSN: 0261-4189. Pub. country: England: United Kingdom. Language: English.

AB **Platelet** adhesion on and activation by components of the extracellular matrix are crucial to arrest post-traumatic bleeding, but can also harm tissue by occluding diseased vessels. Integrin alpha2beta1 is thought to be essential for **platelet** adhesion to subendothelial collagens, facilitating subsequent interactions with the activating **platelet** collagen receptor, **glycoprotein VI** (**GPVI**). Here we show that Cre/loxP-mediated loss of beta1 integrin on **platelets** has no significant effect on the bleeding time in mice. Aggregation of beta1-null **platelets** to native fibrillar collagen is delayed, but not reduced, whereas aggregation to enzymatically digested soluble collagen is abolished. Furthermore, beta1-null **platelets** adhere to fibrillar, but not soluble collagen under static as well as low (300 s(-1)) and high (1500 s(-1)) shear flow conditions, probably through binding of alpha(IIb)beta3 to von Willebrand factor. On the other hand, we show that **platelets** lacking **GPVI** can not activate integrins and consequently fail to adhere to and aggregate on fibrillar as well as soluble collagen. These data show that **GPVI** plays the central role in **platelet**-collagen interactions by activating different adhesive receptors, including alpha2beta1 integrin, which strengthens adhesion without being essential.

L6 ANSWER 11 OF 21 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
2001406134 EMBASE Bilinexin, a snake C-type lectin from Agkistrodon bilineatus venom agglutinates **platelets** via GPIb and alpha.2 beta.1. Du X.-Y.; Navdaev A.; Clemetson J.M.; Magnenat E.; Wells T.N.C.; Clemetson K.J.. Dr. K.J. Clemetson, Theodor Kocher Institute, University of Berne, Freie Strasse 1, CH-3012 Berne, Switzerland. clemetson@ktk.mlbe.ch. Thrombosis and Haemostasis 86/5 (1277-1283) 2001.
Refs: 25.
ISSN: 0340-6245. CODEN: THHADQ. Pub. Country: Germany. Language: English. Summary Language: English.

AB A new snake protein, named bilinexin, has been purified from Agkistrodon bilineatus venom by ion-exchange chromatography and gel filtration chromatography. Under non-reducing condition it has a mass of 110 kDa protein on SDS-PAGE. On reduction, it can be separated into five subunits with masses in the range 18-28 kDa. The N-terminal sequences of these subunits are very similar to those of convulxin or the alboaggregins, identifying bilinexin as a new member of the snake C-type lectin family, unusual in having multiple subunits. Bilinexin agglutinates fixed **platelets**, washed **platelets** and **platelet** rich plasma (PRP) without obvious activation (shape change) as confirmed by light microscope examination. Both inhibitory and binding studies indicate that **antibodies** against alpha.(2 beta.1) inhibit not only **platelet** agglutination induced by bilinexin, but also bilinexin binding to **platelets**. VM16d, a **monoclonal** anti-GPIb alpha. **antibody**, completely inhibits **platelet** agglutination induced by bilinexin, and polyclonal **antibodies** against GPIb alpha. prevent its binding to **platelets**. However, neither convulxin, polyclonal anti-**GPVI antibodies**, nor GPIIb/IIIa inhibitors affect its binding to and agglutination of **platelets**. Bilinexin neither activates GPIIb IIIa integrin on **platelets** nor induces tyrosine phosphorylation of **platelet**

proteins, nor increases intracellular Ca(2+) in **platelets**. Like alboaggregin B, bilitrexin agglutinates **platelets**, which makes it a good tool to investigate the differences in mechanism between snake C-type lectins causing **platelet** agglutination and those that induce full activation.

L6 ANSWER 12 OF 21 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:161483 Document No.: PREV200200261401. Expression of **GPVI** alone confers collagen signaling in RBL-2H3 cells but inactivation of both **GPVI** and alpha2beta1 is required to inhibit the collagen response of human **platelets**. Chen, Hong (1); Locke, Darren (1); Liu, Changqing (1); Liu, Ying (1); Kahn, Mark L. (1). (1) Molecular Cardiology, University of Pennsylvania, Philadelphia, PA USA. Blood, (November 16, 2001) Vol. 98, No. 10 Part 1, pp. 786a-787a. http://www.bloodjournal.org/. print. Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 1. Orlando, Florida, USA December 7-11, 2001 ISSN: 0006-4971. Language: English.

AB The responses of **platelets** to collagen are primary events in arterial thrombosis and are believed to be mediated by two receptors, **GPVI**-Fc Fcgamma and the integrin alpha2beta1. To determine the role of human **GPVI** we have expressed **GPVI** in RBL-2H3 cells, a mast cell line which expresses abundant Fc Rgamma but no known collagen receptors, and developed blocking **monoclonal antibodies** to human **GPVI**. These experiments revealed for the first time that RBL-2H3 cells expressing high levels of **GPVI** are capable of both adhesion and calcium signaling in response to fibrillar collagen. Cells expressing lower levels of **GPVI** exhibited adhesion but not signaling, or failed to respond to collagen. Quantitation of **GPVI** receptor density on the surface of **GPVI**-expressing RBL-2H3 cells using 125I-labelled anti-**GPVI monoclonal antibody** revealed that the **GPVI** receptor density on high expressing clones is equivalent to that found in human **platelets** (approximately 1400 receptors/**platelet**). To test whether **GPVI** is required for collagen responses in human **platelets** we developed a **monoclonal antibody**, 1A12, which blocks calcium signaling in response to collagen but not the **GPVI** agonist convulxin in RBL-2H3 cells. 30 mug/ml 1A12 had a small inhibitory effect on **platelet** aggregation induced by low (1 mg/ml) but not high concentrations of collagen (10 and 30 mug/ml). A similar small inhibitory effect was observed with the alpha2beta1-blocking **antibody** 6F1 used at the same concentration. Strikingly, a combination of 1A12 and 6F1 virtually ablated **platelet** aggregation in response to collagen (10 and 30 mug/ml). Our results suggest that (1) **GPVI** is sufficient for both adhesive and signaling responses to collagen; (2) **GPVI**-mediated collagen responses are receptor-density dependent; (3) inhibition of collagen stimulated aggregation of human **platelets** requires inhibition of both **GPVI** and alpha2beta1. Experiments are currently underway to determine whether the synergistic effect of blocking both alpha2beta1 and **GPVI** is due to inhibition of alpha2beta1-dependent collagen interaction with **GPVI**, **GPVI**-dependent activation of the alpha2beta1 integrin or to simultaneous inhibition of intracellular signaling by both receptors.

L6 ANSWER 13 OF 21 MEDLINE DUPLICATE 8
2001215216 Document Number: 21215216. PubMed ID: 11181698. Long-term antithrombotic protection by in vivo depletion of **platelet** **glycoprotein VI** in mice. Nieswandt B; Schulte V; Bergmeier W; Mokhtari-Nejad R; Rackebrandt K; Cazenave J P; Ohlmann P; Gachet C; Zirngibl H. (Department of Molecular Oncology, General Surgery, Witten/Herdecke University, 42117 Wuppertal, Germany.. nieswand@klinikum-wuppertal.de) . JOURNAL OF EXPERIMENTAL MEDICINE, (2001 Feb 19) 193 (4) 459-69. Journal code: 2985109R. ISSN: 0022-1007. Pub.

country: United States. Language: English.

AB Coronary artery thrombosis is often initiated by abrupt disruption of the atherosclerotic plaque and activation of **platelets** on the subendothelial layers in the disrupted plaque. The extracellular matrix protein collagen is the most thrombogenic constituent of the subendothelial layer; therefore, a selective inhibition of the collagen activation pathway in **platelets** may provide strong antithrombotic protection while preserving other **platelet** functions. Here we demonstrate that treatment of mice with a **monoclonal antibody** against the activating **platelet** collagen receptor **glycoprotein VI** (**GPVI**; JAQ1) results in specific depletion of the receptor from circulating **platelets** and abolished responses of these cells to collagen and collagen-related peptides (CRPs). JAQ1-treated mice were completely protected for at least 2 wk against lethal thromboembolism induced by infusion of a mixture of collagen (0.8 mg/kg) and epinephrine (60 microg/ml). The tail bleeding times in JAQ1-treated mice were only moderately increased compared with control mice probably because the treatment did not affect **platelet** activation by other agonists such as adenosine diphosphate or phorbol myristate acetate. These results suggest that **GPVI** might become a target for long-term prophylaxis of ischemic cardiovascular diseases and provide the first evidence that it is possible to specifically deplete an activating glycoprotein receptor from circulating **platelets** in vivo.

L6 ANSWER 14 OF 21 MEDLINE DUPLICATE 4
2001113665 Document Number: 20576376. PubMed ID: 11036073. Evidence for two distinct epitopes within collagen for activation of murine **platelets**. Schulte V; Snell D; Bergmeier W; Zirngibl H; Watson S P; Nieswandt B. Department of Molecular Oncology, General Surgery, Witten/Herdecke University, 42117 Wuppertal, Germany. JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Jan 5) 276 (1) 364-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB It has recently been shown that the **monoclonal antibody** JAQ1 to murine **glycoprotein VI** (**GPVI**) can cause aggregation of mouse **platelets** upon **antibody** cross-linking and that collagen-induced **platelet** aggregation can be inhibited by preincubation of **platelets** with JAQ1 in the absence of cross-linking (Nieswandt, B., Bergmeier, W., Schulte, V., Rackebrandt, K., Gessner, J. E., and Zirngibl, H. (2000) J. Biol. Chem. 275, 23998-24002). In the present study, we have shown that cross-linking of **GPVI** by JAQ1 results in tyrosine phosphorylation of the same profile of proteins as that induced by collagen, including the Fc receptor (FcR) gamma-chain, Syk, LAT, SLP-76, and phospholipase C gamma 2. In contrast, **platelet** aggregation and tyrosine phosphorylation of these proteins were inhibited when mouse **platelets** were preincubated with JAQ1 in the absence of cross-linking and were subsequently stimulated with a collagen-related peptide (CRP) that is specific for **GPVI** and low concentrations of collagen. However, at higher concentrations of collagen, but not CRP, aggregation of **platelets** and tyrosine phosphorylation of the above proteins (except for the adapter LAT) is re-established despite the presence of JAQ1. These observations suggest that a second activatory binding site, which is distinct from the CRP binding site on **GPVI** on mouse **platelets**, is occupied in the presence of high concentrations of collagen. Although this could be a second site on **GPVI** that is activated by a novel motif within the collagen molecule, the absence of LAT phosphorylation in response to collagen in the presence of JAQ1 suggests that this is more likely to be caused by activation of a second receptor that is also coupled to the FcR gamma-chain. The possibility that this response is mediated by a receptor that is not coupled to FcR gamma-chain is excluded on the grounds that aggregation is absent in **platelets** from FcR gamma-chain-deficient mice.

L6 ANSWER 15 OF 21 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:129535 Document No.: PREV200200129535. The **platelet** collagen receptor **glycoprotein VI** (**GPVI**) signals through lipid rafts in a Fc Rgamma-dependent manner. Locke, Darren (1); Chen, Hong (1); Liu, Chang-Dong (1); Kahn, Mark L. (1). (1) Molecular Cardiology, University of Pennsylvania, Philadelphia, PA USA. Blood, (November 16, 2001) Vol. 98, No. 11 Part 1, pp. 125a. http://www.bloodjournal.org/. print. Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001 ISSN: 0006-4971. Language: English.

AB The **platelet** collagen receptor **GPVI** signals through the immunoreceptor tyrosine activation motif (ITAM) of its co-receptor Fc Rgamma using many of the same downstream signaling proteins as T cell, B cell and Fc receptors. Signaling by these immune receptors is believed to proceed from receptor clustering to ITAM tyrosine phosphorylation by the src family kinases Fyn and Lyn and subsequent activation of the tyrosine kinases Syk or ZAP-70. Activation of immune receptors results in receptor movement to cholesterol-rich areas of the cell membrane known as lipid rafts that are enriched in Fyn, Lyn and the transmembrane adaptor protein LAT and are defined by their resistance to solubilization by nonionic detergents. To determine whether activation of **GPVI** results in receptor movement to lipid rafts, we expressed **GPVI** in RBL-2H3 cells, a mast cell line which expresses abundant Fc Rgamma but no known collagen receptors. Activation of **GPVI** with the agonist convulxin resulted in a rapid, transient movement of **GPVI** receptors to lipid rafts, a response which was also seen with activation of endogenous Fc epsilon receptors which also couple to Fc Rgamma. The mechanism by which immune receptor activation results in receptor movement to lipid rafts is unknown. To determine the contribution of Fc Rgamma for **GPVI** movement to lipid rafts we examined the behavior of **GPVI** R27L, a previously characterized mutant **GPVI** receptor in which a single amino acid substitution results in loss of Fc Rgamma coupling and intracellular signaling despite normal surface expression. **GPVI** R27L binds CVX but does not move to lipid rafts following ligand binding, suggesting that **GPVI** receptor movement to lipid rafts is mediated by the Fc Rgamma chain. The role of lipid rafts in **platelet** signaling by **GPVI** and other receptors has not been defined. Using a novel anti-**GPVI** **monoclonal antibody**, HY101, we have isolated lipid rafts from human **platelets** and shown that, like **GPVI** -expressing RBL-2H3 cells, **platelet** stimulation of **GPVI** by convulxin results in the transient movement of **GPVI** to lipid rafts. Our results demonstrate that, during **GPVI** signaling the receptor moves to lipid rafts in both RBL-2H3 cells and in human **platelets**, and that **GPVI** movement to lipid rafts following ligand binding is driven by associated Fc Rgamma chain and is not a simple consequence of ligand-induced receptor clustering. Studies are presently underway to determine whether **GPVI**-Fc Rgamma movement to lipid rafts is required for ITAM phosphorylation or vice-versa and to better define the role of lipid rafts for signaling by collagen in human **platelets**.

L6 ANSWER 16 OF 21 MEDLINE DUPLICATE 10
2000423[illegible] Document Number: 20379304. Pubmed ID: 10825177. Expression and function of the mouse collagen receptor **glycoprotein VI** is strictly dependent on its association with the FcRgamma chain. Nieswandt B; Bergmeier W; Schulte V; Rackebrandt K; Gessner J E; Zirngibl H. (Department of Molecular Oncology, General Surgery, University of Witten-Herdecke, 42283 Wuppertal, Germany.. nieswand@klinikum-wuppertal.de) . JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Aug 4) 275 (31) 23998-4002. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB **Platelet** glycoprotein (GP) VI has been proposed as the major collagen receptor for activation of human **platelets**. Human **GPVI** belongs to the immunoglobulin superfamily and is noncovalently associated with the FcRgamma chain that is involved in signaling through the receptor. In mice, similar mechanisms seem to exist as **platelets** from FcRgamma chain-deficient mice do not aggregate in response to collagen. However, the activating collagen receptor on mouse **platelets** has not been definitively identified. In the current study we examined the function and in vivo expression of **GPVI** in control and FcRgamma chain-deficient mice with the first **monoclonal antibody** against **GPVI** (JAQ1). In wild type **platelets**, JAQ1 inhibited **platelet** aggregation induced by collagen but not PMA or thrombin. Cross-linking of bound JAQ1, on the other hand, induced aggregation of wild type but not FcRgamma chain-deficient **platelets**. JAQ1 stained **platelets** and megakaryocytes from wild type but not FcRgamma chain deficient mice. Furthermore, JAQ1 recognized **GPVI** (approximately 62 kDa) in immunoprecipitation and Western blot experiments with wild type but not FcRgamma chain deficient **platelets**. These results strongly suggest that **GPVI** is the collagen receptor responsible for **platelet** activation in mice and demonstrate that the association with the FcRgamma chain is critical for its expression and function.

L6 ANSWER 11 OF 21 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:611992 Document No.: PREV200110851654. Long-term antithrombotic protection by irreversible inactivation of **platelet** **glycoprotein VI** in mice. Nieswandt, Bernhard (1); Schulte, Valerie (1); Bergmeier, Wolfgang (1); Mokhtari-Nejad, Rabee (1); Rackwitz, Jean P.; Schmet, Christian; Zirngibl, Hubert (1). (1) Molecular Oncology, Witten Herdecke University, Wuppertal Germany. Blood, (November 16, 2001) Vol. 98, No. 11 Part 1, pp. 269a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01, 2000 American Society of Hematology. ISSN: 0006-4971. Language: English. Summary Language: English.

AB Coronary artery thrombosis is often initiated by abrupt disruption of the atherosclerotic plaque followed by deposition and activation of **platelets** on the subendothelial layers in the disrupted plaque. Because the extracellular matrix protein collagen is the most thrombogenic constituent of the subendothelial layer, a selective inhibition of the collagen activation pathway in **platelets** may provide strong antithrombotic protection while preserving other **platelet** functions. Growing evidence suggests that **platelet** glycoprotein GP VI is the major collagen receptor for **platelet** activation making this receptor a good candidate for such a specific inhibition. In the current study, we have investigated the antithrombotic effects of the first **monoclonal antibody** mAb against mouse **GPVI** (JAQ1, Nieswandt et al; 2001, J Biol Chem, 276(31):28823-28832). Injection of 100 mug JAQ1 only had mild and transient effects on **platelet** counts with a maximum drop of approximately 64 +- 7.1 % on day 1 and a return to normal after 2-3 days. JAQ1 pretreated mice were completely protected against lethal thromboembolism induced by infusion of a mixture of collagen (0.8 mg/kg) and epinephrine (60 mug/kg) for at least two weeks (100% survivors on days 1, 7, and 14 after mAb injection, n=8 per group, 5% survivors in the control group, n=20). Aggregometric and flow cytometric studies demonstrated that **platelets** from JAQ1 treated mice were completely resistant against activation with high concentrations of collagen (up to 30 mug/ml) and collagen related peptides (up to 100 mug/ml) ex vivo on days 1, 7, and 14. In JAQ1 treated mice, **GPVI** was not detectable in a Western blot analysis of **platelet** lysates for minimally two weeks, suggesting irreversible inactivation (or degradation) of the receptor on circulating **platelets**. In

contrast to collagen, other agonists, such as ADP or **platelet** aggregating agents, such as PMA induced normal activation and aggregation of these **platelets**. Consequently, the tail bleeding times were only moderately increased in anti-**GPVI** treated mice compared to control mice on day 3, 7, and 14. These results establish **GPVI** as an attractive target for long-term antithrombotic therapy.

L6 ANSWER 18 OF 31 MEDLINE DUPLICATE 11
1999167243 Document Number: 99167143. PubMed ID: 10066433. Signal transduction pathways mediated by glycoprotein Ia/IIa in human **platelets**: comparison with those of **glycoprotein VI**. Inoue K; Ozaki Y; Satoh K; Wu Y; Yatomi Y; Shin Y; Morita T.
Department of Clinical and Laboratory Medicine, Yamanashi Medical University, Shimokato 1110 Tamaho, Yamanashi, Nakakoma, 409-3898, Japan.
BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1999 Mar 5) 256 (1) 114-20. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.
AB Human **platelets** were activated either by glycoprotein (GP) Ia/IIa agonist (rhodocytin) or by a **GPVI** agonist (collagen-related peptide, CRP), and the intracellular signal transduction pathways were compared in the presence of various inhibitors. Rhodocytin isolated from Calloselasma rhodostoma venom was verified as a GPIa/IIa agonist, based on the inhibitory effects of three mAbs directed against GPIa. **Platelet** activation mediated by GPIa/IIa led to overt **platelet** aggregation, elevation of intracellular Ca2+, and tyrosine phosphorylation of several proteins, similar to that of **GPVI**. p72 syk and phospholipase Cgamma2 (PLCgamma2) tyrosine phosphorylation were also observed with GPIa/IIa-mediated **platelet** aggregation, although they peaked slightly later than that of **GPVI**. In contrast to **GPVI**-mediated **platelet** activation, most of these phenomena induced by GPIa/IIa activation were markedly suppressed by acetylsalicylic acid (ASA) or cytochalasin D. These findings suggest that the requirements for thromboxane A2 (TXA2) production and actin polymerization, which are the characteristics of collagen-induced **platelet** activation, are derived from the GPIa/IIa-mediated signal transduction, but not from that of **GPVI**.

L6 ANSWER 19 OF 31 SCISEARCH COPYRIGHT 2002 ISI (R)
1998:366552 The Genuine Article (R) Number: ZM110. Simple collagen-like peptides support **platelet** adhesion under static but not under flow conditions: Interaction via alpha beta 1 and von Willebrand factor with specific sequences in native collagen is a requirement to resist shear forces. Verkleij M W (Reprint); Morton L F; Knight C G; deGroot P G; Barnes M J; Sixma J J. UNIV UTRECHT HOSP, DEPT HAEMATOL, POSTGRADUAL SCH BIOMEMBRANES, POB 85500, NL-3508 GA UTRECHT, NETHERLANDS (Reprint); STRANGEWAYS RES LAB, CAMBRIDGE CB1 4RN, ENGLAND. BLOOD (15 MAY 1998) Vol. 91, No. 10, pp. 3808-3816. Publisher: W B SAUNDERS CO. INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399. ISSN: 0006-4971. Pub. country: NETHERLANDS; ENGLAND. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB The aim of this study was to define the need for specific collagen sequences and the role of their conformation in **platelet** adhesion to collagen under both static and flow conditions. We recently reported that simple triple-helical collagen-related peptides (CRPs), GCP*(GPP*)(10)GCP*G and GKP*(GPP*)(10)GKP*G (single-letter amino acid code, P* = hydroxyproline; Morton et al, Biochem J 306:337, 1995) were potent stimulators of **platelet** activation and were able to support the adhesion of gel-filtered **platelets** examined under static conditions. The present study investigated whether these same peptides were able to support **platelet** adhesion under more physiologic conditions by examining static adhesion with **platelet**-rich plasma (PRP) and adhesion underflow conditions. In the static

adhesion assay, we observed 20% surface coverage with **platelet** aggregates. In marked contrast, there was a total lack of adhesion under flow conditions examined at shear rates of 50 and 300 s(-1). Thus, the interaction of **platelets** with the CRPs is a low-affinity interaction unable on its own to withstand shear forces. However, the addition of CRPs to whole blood, in the presence of 200 mu mol/L D-arginyl-glycyl-L-aspartyl-L-tryptophan (RGDW) to prevent **platelet** aggregation, caused an inhibition of about 50% of **platelet** adhesion to collagens I and III under flow. These results suggest that the collagen triple helix per se, as defined by these simple collagen sequences, plays an important contributory role in the overall process of adhesion to collagen under flow. The **monoclonal antibody** (MoAb) 6F1, directed against the alpha 2 subunit of the integrin alpha 2 beta 1, was found to inhibit static **platelet** adhesion to monomeric but not fibrillar collagens I and III. However, under flow conditions, anti-alpha 2 MoAbs 6F1 and 6G1 inhibited adhesion to both monomeric and fibrillar collagens, indicating that alpha 2 beta 1 is essential for adhesion to collagen under flow, independent of collagen conformation, whether monomeric or polymeric. To obtain further insight into the nature of the different adhesive properties of CRPs and native collagen, we investigated the relative importance of von Willebrand factor (vWF) and the integrin alpha 2 beta 1 in **platelet** adhesion to collagen types I and III, using the same shear rate (300 s(-1)) as used when testing CRPs under flow conditions. Our results, together with recent data of others, support a two step mechanism of **platelet** interaction with collagen under flow conditions. The first step involves adhesion via both the indirect interaction of **platelet** glycoprotein (GP) Ib with collagen mediated by vWF binding to specific vWF recognition sites on collagen and the direct interaction between **platelet** alpha 2 beta 1 and specific alpha 2 beta 1-recognition sites on collagen. This suffices to bind **platelets** at the collagen surface. The second step occurs via another collagen receptor (thought to be **GPVI**) that binds to simple collagen sequences, required essentially to delineate the collagen triple helix. Recognition of the triple helix leads to strengthening of attachment and **platelet** activation. (C) 1996 by The American Society of Hematology.

L6 ANSWER 20 OF 21 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V. DUPLICATE 12
1998218100 EMBASE Convulxin-induced **platelet** adhesion and aggregation: involvement of **glycoproteins VI** and
Jandrot-Perrus M.; Dr. M. Jandrot-Perrus, Lab. Recherche Hemostase Thrombose, Faculte de Medecine Xavier Bichat, 75870 Paris Cedex 18, France. Platelets (1998) 9:3-4 (207-211)
1998.
Refs: 22.
ISSN: 0953-7104. CODEN: PLTEEP. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB The interaction of convulxin (Cvx), a 72-kDa glycoprotein isolated from the venom of Crotalus durissus terrificus with human **platelets** has been studied. Cvx at low concentrations (below 100 pM) induced **platelet** aggregation, dense body secretion and intracellular calcium mobilization which indicates that Cvx is a potent activator of human **platelets**. Cvx-induced **platelet** aggregation and secretion was inhibited by 6F1 an anti-integrin .alpha.2.beta.1 **monoclonal antibody** that was without effect on calcium mobilization. Anti-**GPVI** Fab fragments inhibited aggregation, secretion and calcium mobilization triggered by Cvx. In addition, immobilized Cvx was found to induce divalent cation-independent **platelet** adhesion in a static system. **Platelet** adhesion to Cvx was inhibited by anti-**GPVI** Fab fragment but not by anti-integrin .alpha.2.beta.1. Cvx was shown to bind to a 57,000 Dalton protein that was identified as **GPVI**. Altogether, these results

indicate that **GPVI** behaves as a receptor for Cvx, while integrin .alpha.2.beta.1 could play a regulatory role in Cvx-induced **platelet** aggregation. Cvx and collagen interaction with **platelets**, thus appears to share some characteristics but to also have specific properties.

L6 ANSWER 21 OF 21 MEDLINE DUPLICATE 13
1998001677 Document Number: 98001677. PubMed ID: 9841142. Adhesion and activation of human **platelets** induced by convulxin involve **glycoprotein VI** and integrin alpha2beta1. Jandrot-Perrus M; Lagrue A H; Okuma M; Bon C. (Laboratoire de Recherche sur l'Hemostase et la Thrombose, Faculte de Medecine Xavier Bichat, BP 416, 75870 Paris Cedex 18, France.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Oct 24) 272 (43) 27105-41. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB We analyzed the interaction of convulxin (Cvx), a 72 kDa protein isolated from the venom of Crotalus durissus terrificus, with human **platelets**. Cvx is a potent **platelet** agonist that induces an increase in the intracellular Ca2+ concentration ([Ca2+]i), granule exocytosis and aggregation. 125I-labeled Cvx binds specifically and rapidly to **platelets** at binding sites of high and moderate affinity. **Platelets** adhere to immobilized Cvx in a time-dependent but cation-independent manner. **Platelet** exocytosis and aggregation induced by Cvx were inhibited by an anti-integrin alpha2beta1 **monoclonal antibody** (6F1) and by the Fab fragments of a polyclonal anti-**glycoprotein VI** (**GPVI**) **antibody**. Both the adhesion of **platelets** to Cvx and the Cvx-induced increase in [Ca2+]i were inhibited by anti-**GPVI** Fab fragments but not by 6F1. Ligand blotting assay showed that 125I-Cvx binds to a 57-kDa **platelet** protein with an electrophoretic mobility identical to that of **GPVI**. In addition, we observed the following: (i) 125I-Cvx binds to **GPVI** immunoprecipitated by the anti-**GPVI** **antibody** from a **platelet** lysate, and (ii) Cvx inhibits the binding of anti-**GPVI** IgG to **GPVI**. Taken together, these results demonstrate that **GPVI** behaves as a Cvx receptor and that the alpha2beta1 integrin appears to be involved in the later stages of Cvx-induced **platelet** activation, i.e. exocytosis and aggregation.

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